

## Update Le point

Articles in the *Update* series give a concise, authoritative, and up-to-date survey of the present position in the selected fields, covering many different aspects of the biomedical sciences and public health. Most of the articles are written, by invitation, by acknowledged experts on the subject.

Les articles de la rubrique *Le point* fournissent un bilan concis et fiable de la situation actuelle dans les domaines considérés, couvrant de nombreux aspects des sciences biomédicales et de la santé publique. La plupart de ces articles auront donc été rédigés sur demande par les spécialistes les plus autorisés.

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# Male fertility regulation: recent advances\*

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*Acceptable antifertility drugs for men are proving difficult to produce. Such drugs must aim to achieve complete azoospermia over a long period. This requirement may be relaxed only if it can be shown that the residual sperm produced by men whose spermatogenesis has been suppressed by antifertility drugs to oligospermia are incapable of fertilizing ova. Hormonal methods involving suppression of the secretion of gonadotrophin hormones by the pituitary gland invariably require androgen supplementation, and the use of steroids either alone or in combination requires careful monitoring for their side-effects. A chemical (non-hormonal) approach involving the incapacitation of sperm in the epididymis has been shown to be feasible in animal studies using  $\alpha$ -chlorohydrin and 6-chloro-6-deoxy sugars, although such compounds cannot be developed for human use because of their toxicity. Immunological approaches have the inherent problem of delivery of the antibody to the target. While the search for new and safer chemical and hormonal approaches goes on, the recent evidence that vasectomy offers a safe surgical option leaves responsible men with some choice to add to the condom.*

## INTRODUCTION

In the quest for appropriate family size, it is surely axiomatic that the male partner should be able to share the benefits and risks of whatever contraceptive strategy the couple may follow. Women, however, may argue that a proper sharing will not occur until men everywhere demonstrate a greater readiness to accept their responsibility. At present, nearly 50 million men in the world have opted for vasectomy, and, with growing evidence that there are no long-term deleterious effects from this operation (1, 2), more and more men are choosing this surgical option (see 3, for example).

While vasectomy can be reversed by skilful surgeons with some success in terms of recovery of patency (2), it is not offered as a reversible procedure. Thus, for men ultimately to have the same choice as women, there is clearly a need for a safe antifertility pill

\* A French translation of this article will appear in a later issue of the *Bulletin*.

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which should be capable of reversibly suppressing sperm production or sperm function without interfering with the libido or any other short- or long-term features of the health status of men. In spite of a number of exciting developments in recent years, in which, since 1971, the WHO Special Programme of Research, Development and Research Training in Human Reproduction has played a key role through its Task Force on Methods for the Regulation of Male Fertility, it must be admitted that there is still a long way to go before a chemical or hormonal male pill becomes available.

However, there are signs that the scene may be set for the start of an interesting phase. Several agencies concerned with population studies have maintained a broad involvement in male fertility studies. This work includes research and development of new methods based on hormonal interventions to suppress spermatogenesis, and chemical approaches to interfere with sperm maturation. In addition, work on gossypol and on the safety and efficacy of vasectomy has been conducted. These four subjects are reviewed below.

## HORMONAL SUPPRESSION OF SPERMATOGENESIS

The endocrine suppression of sperm production is difficult to achieve without simultaneously suppressing the production of androgens, which have an important role in erythropoiesis, protein anabolism, bone metabolism, and secondary sex and behavioural characteristics as well as libido and potency. Thus unless spermatogenesis can be suppressed without interfering with steroidogenesis by the Leydig cells, or unless the anti-fertility agent itself is androgenic, such methods will require androgen supplementation. Moreover, this supplementation should lead to recovery of the systemic androgen-dependent functions without locally restimulating spermatogenesis. Finally, any strategy based on suppression of sperm production must aim at consistent long-term azoospermia that could be reversed when desired. This requirement may be relaxed only if it can be shown that the residual sperm produced by men whose spermatogenesis has been suppressed to a level of severe oligospermia were non-functional. Thus the definition of appropriate *in vitro* tests of sperm function is an important component of the research in this field (4).

### Selective inhibition of follicle-stimulating hormone (FSH or follitropin)

In the belief that selective elimination of FSH bioactivity would lead to selective suppression of spermatogenesis without influencing androgen production, strategies to neutralize FSH have been developed based on two approaches: active immunization against FSH; and the isolation of "inhibin", a peptide believed to be secreted by the Sertoli cells and to act by specifically suppressing the secretion of FSH by the pituitary gland.

In the first approach, the active immunization of bonnet monkeys against FSH suggested that reversible FSH suppression would be a viable option (5). Nevertheless, in a long-term study active immunization of rhesus monkeys against FSH for over 4 years failed to suppress spermatogenesis to azoospermia (6). Thus, although no adverse side-effects of immunization occurred, and androgen production remained unaffected, it was apparent that selective inhibition of FSH alone would be insufficient to suppress spermatogenesis in non-human primates. Experiments in hypophysectomized and pituitary-stalk-sectioned monkeys suggest that this is because testosterone alone can maintain qualitative, if not normal quantitative, spermatogenesis.

A major effort to isolate "inhibin" of testicular origin, which was promoted by the

WHO Task Force in the period 1973–79, was also based on the premise that specific inhibition of FSH would arrest spermatogenesis. A number of proteins of defined relative molecular mass ( $M_r$ ) were isolated and purified from testicular tissue or from testicular lymph or fluid (7). While some of these fractions could suppress the secretion of FSH by pituitary tissue in *in vitro* culture systems, none so far has elicited sufficiently consistent biological activity *in vivo* to inhibit spermatogenesis. Very recently, several research groups have claimed major advances in this field. All have used follicular fluid as their source material for “inhibin” peptides but only two groups appear able to claim consistent biological activity for a purified “inhibin” of  $M_r$  56 000, a glycoprotein of two subunits (8). If this research yields a protein of small  $M_r$  with a consistently demonstrable suppressive action on FSH, genetic engineering could possibly yield a sufficiently potent hormone for drug development.<sup>a</sup> Even if this were achieved, the problems of the appropriate means of long-term administration of a peptide substance would remain.

### **Inhibition of gonadotrophin secretion by steroids**

The simultaneous suppression of both gonadotrophin hormones, i.e., FSH and luteinizing hormone (LH, lutropin) by means of estrogens, androgens, and progestogens remains a possibility for male fertility control. Estrogens will suppress spermatogenesis quite effectively, but even with the addition of androgens, feminizing symptoms such as gynaecomastia have remained as a risk.

Exogenous testosterone alone administered as one of several esters, i.e., testosterone propionate, cypionate or enanthate, has proved to be effective in reversibly suppressing sperm production to azoospermic levels in most of the volunteers in several clinical trials (9, 10). A four-centre study conducted by the National Institutes of Health in the USA addressed the two major concerns for the use of the most long-acting ester available, testosterone enanthate, i.e., the frequency with which intramuscular injections must be given and the possible side-effects. The study concluded that injections once per week of 250 mg testosterone enanthate were needed to attain azoospermia or severe oligospermia and although severe side-effects did not appear, some weight gain, decrease in testis size, acne, oily skin, or slightly increased haemoglobin concentrations were observed (11).

A 5-country acceptability study conducted by the WHO human reproduction programme in 1976 showed that while the monthly administration of an antifertility agent to men by injection was not unacceptable, most of the men stated that they preferred a pill to the injection as a route of administration. However, none of the orally active androgens available has proved to be effective in uniformly suppressing spermatogenesis. The reason for this appears to be related to poor pharmacokinetics. For example, relatively short-lived peaks of testosterone appear 1–8 hours after oral doses of testosterone undecanoate. The synthetic oral androgens, e.g., fluoxymesterone and mesterolone, were investigated by the pharmaceutical industry but found to be ineffective in suppressing spermatogenesis, and methyltestosterone is prohibited because of potential liver toxicity.

Cyproterone, an antiandrogen, has been used in the treatment of men diagnosed as sexual offenders and it was anticipated that, administered as the acetate ester, it would inhibit spermatogenesis through the suppression of pituitary gonadotrophin secretion. In WHO-supported clinical trials, daily oral administration of 5–20 mg/day for up to 16 weeks drastically reduced sperm counts and motility and induced infertility. However, the trials were discontinued because of the consequent androgen deprivation. The combination of cyproterone with a long-acting androgen is likely to be a future strategy when the latter becomes available.

<sup>a</sup> Note added in proof. New work on the cloning and sequence analysis of cDNA coding for the  $\alpha$ - and  $\beta$ -chains of porcine inhibin (20) and bovine inhibin (21) takes the possibility of the genetic engineering of inhibin a major step forward.

19-Nortestosterone (19NT) has both anabolic and androgenic potency and has been in clinical use for almost two decades without serious side-effects. Recently, a German group conducted clinical trials with 19-NT and 19-NT cyclohexylphenylpropionate, an ester with a long half-life. 19-Nortestosterone injected intramuscularly into men at 1–3-week intervals for 25 weeks suppressed plasma LH and FSH concentrations to below detectable levels and plasma testosterone declined to castrate ranges without changes in libido or potency or in serum lipids; a slight increase in haemoglobin and erythrocyte volume fraction (haematocrit) was noted. Azoospermia or severe oligospermia was achieved in most subjects (12). This promising result is being followed up, although 19-nortestosterone may not be better than testosterone itself as a single injectable steroid.

Combinations of two steroids have been studied in clinical trials, e.g., danazol (a derivative of ethinyl testosterone) and various progestogens for the suppression of pituitary secretory function combined with testosterone esters for androgen replacement. These have induced reversible azoospermia or severe oligospermia without serious side-effects. While the results are encouraging, a combination of two steroids with such radically different pharmacokinetic reactions is likely to lead to the lack of uniformity in the responses that have been observed and also to raise serious concerns about the effects on liver function from long-term use with the administration of 600–800 mg doses of steroid per day.

Different progestogens have been used in combination with testosterone in clinical trials for male fertility control: norethisterone, medroxyprogesterone acetate (MPA), and depot-MPA (DMPA), 17-hydroxyprogesterone capronate, and megestrol acetate. The most favourable combination emerging from the trials is the monthly intramuscular injection of 200 mg DMPA plus 200 mg testosterone enanthate or testosterone cypionate. This combination showed the best results in suppressing spermatogenesis and the incidence of untoward side-effects was low. No effects on libido and potency were observed. However, even this combination did not produce azoospermia uniformly, so that its efficacy remains uncertain (13).

In order to develop a regimen that could be self-administered, French groups have administered medroxyprogesterone acetate or norethisterone orally plus testosterone or dihydrotestosterone given percutaneously by cream. In the most successful trials, sperm counts were suppressed to azoospermia or severe oligospermia. Unfortunately, some of the wives developed androgenization from the testosterone cream transferred from their treated husbands (14). It is evident that one of the major advances in the field would be the development of a much longer-acting androgen hormone yielding a continuous slow release of testosterone over a period of several months. Several long-acting testosterone esters have been synthesized in a WHO steroid synthesis programme and are being developed for both clinical and antifertility uses.

### **Gonadotrophin (luteinizing)-releasing hormone analogues**

Peptides with closely similar structure to that of the gonadotrophin-releasing hormone (LHRH or GnRH), secreted by neurones in the median eminence of the hypothalamus, have been synthesized in recent years and shown to have two major effects, either as GnRH *agonists* or as GnRH *antagonists*. Several pharmaceutical companies have also developed GnRH analogues and have used them mainly for gonadotrophin and steroidogenic suppression in the treatment of cancer of the prostate and precocious puberty. Both agonists and antagonists have been explored for their ability to inhibit testicular function through the suppression of gonadotrophin secretion. Testosterone supplementation was again found to be essential.

It appears that delivery of the GnRH agonist by constant release, rather than by inter-

mittent injection, is preferable and has been achieved by the use of the extracorporeal mini-osmotic pumps, developed for insulin infusions (15). The GnRH agonist administered in this way caused an initial rise in the plasma levels of LH, FSH and testosterone and then, paradoxically, a decrease to below the normal range. Once again, androgens administered by injection or given orally were needed to offset the consequent androgen deficiency. Even so, the stepwise increases in GnRH administration in successive trials failed to achieve azoospermia at even the highest dose (440  $\mu\text{g/day}$ ) and the possibility emerged that the intermittent injection of testosterone for replacement is counter-productive and may itself sustain spermatogenesis. In one centre, the oral administration of two or three testosterone undecanoate capsules per day produced two or three serum testosterone peaks which might have acted to restimulate spermatogenesis when given sequentially.

Analogues of GnRH, acting as antagonist drugs to achieve their effect by directly blocking GnRH receptors in the pituitary gland, are likely to be more successful than GnRH agonists which act by desensitization following a variable initial degree of pituitary and therefore testis stimulation. Such GnRH antagonists have only recently been produced and two have been studied by constant infusion into cynomolgus monkeys. They caused an immediate and precipitous decline in plasma concentrations of LH and testosterone; testicular volume also decreased and most of the monkeys became azoospermic. The effect was reversible (16). However, some toxicity problems have arisen with GnRH antagonists which involve histamine-induced peripheral oedema. These problems will have to be resolved before clinical trials can be initiated although GnRH antagonists would seem to have better potential for male fertility regulation than GnRH agonists.

Several other issues have to be resolved. The problems with testosterone supplementation will require careful consideration in future clinical trials; the development of an appropriate biodegradable delivery system for these and other peptide-based drugs is needed; and reassurance over the question of recovery of the testis to normal function after long periods of suppression remains to be established.

## CHEMICAL INTERFERENCE WITH SPERM MATURATION

Extensive studies on the function of the epididymis and the nature of the process of sperm maturation within this organ have considerably increased current awareness of the opportunities offered by an antifertility strategy based on chemical, non-hormonal interference in the epididymis. Drug action on sperm after they have left the testis would, ideally, not disturb spermatogenesis and would therefore be likely to have not only a rapid effect but also, on removal, a rapid return of normal sperm to the ejaculate. Unacceptable toxicity removed from consideration two such agents which showed early promise: the S-enantiomer (isomer) of  $\alpha$ -chlorohydrin (3-chloro-1,2-propanediol) and a range of 6-chloro-6-deoxyhexoses and sucroses. These compounds interfered with the glycolytic capability of stored sperm, principally by inhibiting the glyceraldehyde 3-phosphate dehydrogenase enzyme, leading to incapacity of the flagellum and therefore to interference with the motility of the sperms which are rendered infertile (17).

Comparatively recently, salazosulfapyridine (2-hydroxy-5[[4-[(2-pyridinylamino)sulfonyl]phenyl]azo]benzoic acid), a sulfonamide drug in widespread use for the treatment of ulcerative colitis, was shown to cause reversible infertility in the human and the rat (18). Patients on the drug for less than two months produced semen of normal density but with sperm of significantly depressed motility and abnormal morphology. Sperm density

declined during longer-term treatment but the effects were reversible after drug withdrawal. These observations and experiments with rats suggested that the effect may be both on later spermatogenesis and in reducing the motility capacity of sperms in the epididymis. The parent compound undergoes azo-reduction to sulfapyridine and 5-aminosalicylic acid by bacterial action in the large intestine. Most of the sulfapyridine is absorbed and, in rats, this compound caused almost as great an impairment of fertility as the parent compound. While salazosulfapyridine will not itself prove to be an acceptable antifertility compound, it seems to be an example of a drug which had been in clinical use for 40 years before its action on epididymal sperm was recognized.

In recognition of the need for better spermicidal agents, particularly in developing countries, and for chemical agents with an action on epididymal sperm function, the WHO programme on human reproduction is collaborating with the International Organization for Chemical Sciences in Development (IOCD) in a chemical synthesis programme aimed at creating analogues of a range of known and putative drugs for screening of their action on sperm stored in the epididymis. Collaboration with industry is making drugs available for this programme and investigations on the mechanism of access to the epididymis of such agents should also create better screening procedures.

Despite the promise of more rapid action and freedom from androgen suppression offered by such an approach, any newly discovered drugs of this sort, unless they are like salazosulfapyridine with some history of clinical acceptability, would have to enter the long route to establish their safety. This would mean that, even if available now, they would not be on the market for well over a decade.

## GOSSYPOL

Gossypol is a polyphenolic compound (1,1',6,6',7,7'-hexahydroxy-3,3'-dimethyl-5,5'-bis(1-methylethyl)-[2,2'-binaphthalene]-8,8'-dicarboxaldehyde) occurring in the seed and root bark of certain species of cotton plant. In the 1930s in Jiangsu Province in China, the oil popularly used in cooking was changed from soyabean to that of cotton seed origin, whereupon a period of general infertility followed. This was first believed to be of female origin because of the incidence of menstrual disorders, but was later shown to be primarily due to the antispermatogenic effect of gossypol in men. In clinical trials conducted by Chinese doctors, gossypol (given initially as a daily 20 mg pill and then once a week) not only suppressed sperm production to zero in almost all men but also maintained them azoospermic with a consistency not before seen with other drugs (19).

Gossypol is cytotoxic to the germ cells and, in 1-4% of men, induced potassium loss in urine and consequent serum hypokalaemia. In some cases, this was of such severity that neuromuscular disorders arose which, in some, led to transient paralysis. An increasing awareness of the toxicological effects of both hypokalaemia and of the relatively high frequency of irreversible spermatogenic damage induced by gossypol has caused urgent reassessment of its potential as an antifertility agent.

The Rockefeller Foundation has supported limited clinical trials in China and small-scale clinical studies in Brazil and Austria. The dose administered in the current Chinese trial has been reduced from 20 mg to 10-15 mg/day during the loading phase in order to see if severe oligospermia rather than consistent azoospermia would be adequate for an acceptable, non-toxic and reversible effect. Meanwhile, both the WHO human reproduction programme and the Rockefeller Foundation are supporting animal studies to better define the mechanism of action of gossypol. There is also a 13-centre WHO

chemical synthesis programme producing analogues of gossypol that are being tested for pharmaceutical efficacy by the contraceptive development branch of the US National Institutes of Health. Scientists supported by the WHO programme have also separated gossypol into its constituent enantiomers and have shown that the (+) enantiomer is inactive and the (–) enantiomer is the active component. The issue of whether or not toxicity also resides with the (–) enantiomer remains to be explored. If this enantiomer proves to be toxic, then the final hope resides with the synthesis programme producing an active, non-toxic analogue of gossypol.

## VASECTOMY

Recently, two major epidemiological studies have established the safety of vasectomy. A historical cohort study supported by the US National Institutes of Health (1) reported that excess morbidity and mortality occurred in the control population, observations which appear likely to be confirmed by a retrospective follow-up study on vasectomy in Sichuan conducted by Chinese investigators in collaboration with the WHO programme. Thus, much less attention is now given to earlier concerns, in animal studies, that the immune response arising from the release of antigens from sperm in the occluded vas deferens might pose a health hazard.

Vaccination against proteins forming part of the sperm surface membrane is another approach being pursued, but the delivery of antisperm antibody to the target would be difficult in men because of the cell barriers to proteins that surround the sperm ducts. Thus vaccination of women against sperm in the female tract is a more feasible outcome but would not be a truly “male” method.

## CONCLUSION

While research in the field of male fertility regulation is active in several potentially productive areas, no acceptable drug product has yet been produced despite more than a decade of intense effort. Nevertheless, the field is poised at an exciting point although there are clearly special reasons why the need exists for much more fundamental research. Developments in cell and molecular biology and ideas and concepts from neighbouring scientific fields could offer new opportunities for the near future. If one were to speculate, a personal view would be that a chemical pill acting on sperm in the epididymis will emerge as the best hope for the future. Such a pill should not affect the hormonal status of men and would not require androgen supplementation; it should be quick-acting with sure and rapid reversibility.

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